In Utero Transmission of Nipah Virus: Role Played by Pregnancy and Vertical Transmission in Henipavirus Epidemiology

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(See the article by Mungall et al., on pages 812–6.)

How are viruses transmitted within infected organisms? How are they transmitted to other hosts? The answers to these questions are of critical importance for our understanding of viral pathogenesis and viral spread in susceptible animal and human populations. In utero mother-to-fetus transmission of viruses is of particular interest, because it contains elements of both—transmission within an infected host and spread to other hosts. On the one hand, viral spread occurs within the body of the mother through permanent close contact between infected maternal tissue and susceptible fetal tissue. On the other hand, there is a barrier between the mother and fetus that must be crossed by the virus for a successful infection. A complication in any analysis of intrauterine vertical (mother-to-child) transmission is the possibility for infection during delivery, when the barrier is removed and the infant becomes exposed to infected maternal tissue. To distinguish between transmission of virus from mother to fetus in utero versus transmission during delivery or postpartum, specimens from the fetus should be obtained and examined for virus by molecular biological methods and/or histochemical analysis. In many cases, however, only observed pathological changes in the fetus are accepted as evidence for infection of the fetus. By use of either approach, in utero transmission has been established for some viruses, including cytomegalovirus, varicella-zoster virus, rubella virus, poliovirus, Japanese encephalitis virus, coxsackie viruses, echovirus, measles virus, mumps virus, and hepatitis B, C, and E viruses [1, 2]. For other viruses, including HIV, delivery and postpartum transmission are the dominant pathways of vertical transmission. The mechanisms of in utero transmission remain elusive, although it is likely that a major route of transmission from mother to fetus is transplacental and occurs by transcytosis, as has been shown for hepatitis B virus [3, 4]. In most reported cases, infection of the mother results in pathology in the fetus, and the infection of the fetus and the pregnancy of the mother do not significantly affect the course of disease in the mother. However, in some cases pregnancy leads to more-severe disease—for example, in women infected with hepatitis E virus [5]. Thus, in utero transmission of viruses is obviously relevant to human health. Understanding such transmission is also important for improving animal health and reducing the related potential economic impact. An example is the birth of calves with bovine viral diarrhea virus (BVDV) infection as a result of in utero fetal exposure, which is critical to the perpetuation of BVDV in an infected herd and the spread of the virus to other susceptible herds [6]. Furthermore, vertical transmission of henipaviruses in bats, as discussed below, may be an important factor in intermittent henipavirus outbreaks in Southeast Asia and Australia.

Bats—probably the most abundant, diverse, and geographically dispersed vertebrates on earth—have recently been shown to be the reservoir hosts for a variety of zoonotic viruses that are responsible for outbreaks of severe human disease, some with very high mortality. The best studied group of emergent bat zoonotic viruses are the henipaviruses, which include Hendra virus (HeV) and Nipah virus (NiV) [7–18]. HeV emerged in

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Queensland, Australia, in 1994, killing 1 human and 14 horses [19], and it was responsible for at least 4 other sporadic outbreaks involving horses and humans between 1994 and 2006 [17]. The closely related NiV emerged in 1998–1999 in peninsular Malaysia, resulting in the death of >100 people and the culling of >1 million pigs [15]. Since then, several NiV outbreaks have been recorded in Bangladesh and India [17, 20, 21]. During these most recent outbreaks, several important observations have been made—such as the presence of person-to-person transmission, a higher incidence of acute respiratory distress syndrome, and case-fatality rates (60%–75%) higher than those in the Malaysian outbreak (~40%) where the virus is suspected to have originated [22–26].

HeV and NiV are novel members of the family Paramyxoviridae. Paramyxoviruses are negative-sense RNA enveloped viruses and contain 2 major membrane-anchored envelope glycoproteins that are required for infection of a receptive host cell. The broad species tropisms and the ability to cause fatal disease in both animals and humans distinguish HeV and NiV from all other known paramyxoviruses (reviewed in [27]). The substantial differences in their genome sequence and host range led to the establishment of a new genus *(Henipavirus)* in the family to accommodate their taxonomic classification [28, 29]. Fruit bats in the genus *Pteropus* (flying foxes) are the natural reservoir of both HeV and NiV, and NiV is present in fruit bat populations in Indonesia, Thailand, Malaysia, and Cambodia [21].

The exact mechanism of henipavirus transmission from bats to humans is poorly understood. Experimental studies in this area are severely hampered by 2 practical difficulties. First, because bats are wild animals, it is not easy to obtain relevant species of bats that have a known history of being free from prior infection with henipaviruses or closely related viruses. Second, both HeV and NiV are biosafety level (BSL) 4 agents, and any infection studies have to be conducted in BSL4 facilities by skilled, highly trained operators. Nevertheless, on the basis of existing field and laboratory data, 3 hypotheses have been postulated [29]. One is that masticated pellets of virus-contaminated residual fruit pulp spat out by flying foxes are consumed by susceptible animals, such as horses or pigs. The second is that urine from infected animals contaminates pastures or pig sties. The third is that infected fetal tissues or fluids contaminate pastures or sties and are ingested.

The importance of pregnancy and fetal materials in the spread of disease was first hypothesized after the discovery that the index case of the 1994 HeV outbreak was a pregnant mare. This was later supported by the observation that HeV outbreaks have occurred during the birthing period in some species of flying foxes and by the isolation of virus from a pregnant flying fox and its fetus [30]. Vertical transmission of HeV was later experimentally confirmed in guinea pigs and bats [31].

Now, in this issue of the *Journal,* Mungall et al. [32] report the results of a detailed investigation of in utero NiV transmission and provide the first experimental evidence that NiV, like HeV, can be vertically transmitted in cats. Cats are naturally infected and consistently exhibit characteristic disease pathology even at a low MOI [18]. A previous study has demonstrated the presence of HeV antigens both in adult tissues and in the placenta of infected pregnant flying foxes [31]. However, despite rigorous sampling regimens, virus has been isolated only infrequently, and, where isolation was successful, positive sources have included urine and the fetus, heart, placenta, kidney, and spleen of 2 pregnant bats [31]. Mungall et al. isolated significant amounts of infectious NiV from placental fluid (1×10^5 $TCID_{50}$/mL) as well as from placental tissue, although at lower levels from the latter. Evidence was also provided for high levels of viral replication in many tissues of a pregnant adult cat and in fetal tissues, suggesting both vertical and horizontal transmission of this virus—a finding that has important implications for the epidemiology of NiV infection as well as for the testing of inhibitors and vaccines in this animal model and for the understanding of disease mechanisms. The pathology in cats resembled various features seen in humans, although encephalitis has not been observed in cats. Interestingly, temperature increase (as a measure of the development of infection) was initially the same for the pregnant cat and the infected nonpregnant control cat during the first 5 days; this was followed by a rapid increase for the control cat (figure 1 in Mungall et al.), whereas the infected pregnant cat showed a slight drop in temperature followed by a largely constant period until day 12. One can speculate that pregnancy delayed the progression to disease by 1 week, and perhaps the disease progression resumed as a result of the "secondary" infection in the fetus, in which the infection continued unchecked by maternal defense (immune) systems. If this is true, it represents a unique example of protection (although partial in this case) as a result of pregnancy. One can further speculate, as the Mungall et al. did, that such protection could be due to hormonal changes that occur during pregnancy. The data from this study were based only on 2 cats, and more experiments are required to confirm this novel observation.

The results of Mungall et al.'s study have implications for the mechanisms of NiV spread in animal and human populations. The results affirm observations in horses, guinea pigs, and bats that demonstrate that henipaviruses can replicate to high titers in both adult and fetal tissues, indicating that both horizontal and vertical routes of transmission can play a role in spillover events. They also suggest a possible role for cats in HeV and NiV outbreaks that has never been fully investigated, even though cats were observed at the sites of both HeV and NiV outbreaks [33]. Finally, the similarity in major pathological features between this cat model of disease and human infection could help

to develop novel treatments, for example, by identification of maternal pregnancy factors that could delay progression to disease.

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